

Rapid Analysis of Azole Antifungal Drugs in Topical Formulations Using the Hitachi LaChromUltra™ Liquid Chromatography System

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Candida sp., Aspergillus flavus, Tinea pedis, and other fungal infections are common in the environment and health care facilities. These ubiquitous fungal infections in the body can vary from merely an annoyance to life threatening. Fungi can colonize many different areas of the body, and they are often treated with azole antifungals, either topically or orally. Topically dosed antifungals for these conditions are typically formulated into creams, lotions, ointments, or suppositories.

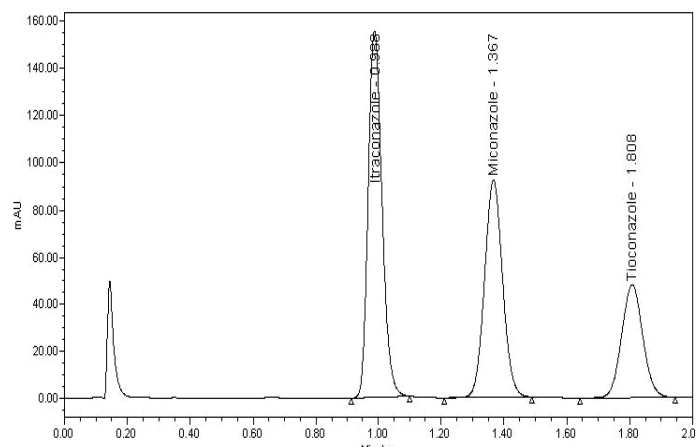
USP Monographs exist which detail the analysis and dosing levels of these antifungal medications. Itraconazole (ITZ), miconazole (MIC), and tioconazole (TIO) have all been used to treat a variety of fungal infections. These compounds, however, have shown long run times with conventional HPLC, especially when analyzing topical formulations containing them. MIC¹, for example, can show a retention time of up to 17 minutes, while ITZ² typically elutes at 22 minutes, and TIO³ can elute at up to 30 minutes under current USP monographic analytical conditions. Using Hitachi's LaChromUltra high-speed chromatographic system, these three compounds (in a single mixture) can be analyzed in under 2 minutes. Isocratic conditions allow for ease of analysis. The Hitachi 2 µm C-18 2.0 x 50 mm column is a rugged reverse phase column which is ideal for analysis of these medications, reducing the overall run time by up to 10-fold.

Experimental Conditions

Module	Conditions and Other
Pump (L-2130U)	35/35/30; CH ₃ CN/ CH ₃ OH / 0.1M NH ₄ C ₂ H ₃ O ₂ in water with 1 mL NH ₄ OH added flow = 0.85 mL/min
Autosampler (L-2200U)	5 µL injection volume, of 0.2 mg/mL each component dissolved in mobile phase, 10°C
Oven (L-2300U)	45°C
UV Detector (L-2455U)	220 nm
Column	Hitachi 2 µm C18 2.0 x 50 mm P/N 891-5000
Samples	ITZ Oral Solution, MIC Vaginal Cream, and TIO Vaginal Ointment

* LaChromUltra is a trademark of Hitachi High Technologies Corporation.

Results



Drug	RT	Area	k'	Tailing	Resolution	Plates
ITZ	0.99	475549	1.5	1.2	-	2450
MIC	1.37	368215	2.4	1.1	4.1	2705
TIO	1.81	223940	3.5	1.0	3.8	3374

Precision: %RSD (n=6)

Compound	Area	Retention Time
ITZ	1.3%	0.4%
MIC	1.6%	0.3%
TIO	0.6%	0.7%

Discussion

Hitachi's LaChromUltra™ Liquid Chromatography System, equipped with a Hitachi 2 µm particle size column, reduces the analysis time for azole antifungals by up to 10-fold. The LaChromUltra is designed to take full advantage of the increased efficiency associated with smaller particle sized columns. These systems allow for reduced production of hazardous waste, higher through-put sample analysis, and less overall organic solvent usage (a major concern in labs), with acceptable precision (>2% RSD).

References:

- 1 - USP 32/NF 27; USP Monograph for Miconazole Vaginal Cream, United States Pharmacopeia, 2009.
- 2 - USP 32/NF 27; USP Monograph for Itraconazole Oral Solution, United States Pharmacopeia, 2009.
- 3 - USP 32/NF 27; USP Monograph for Tioconazole Vaginal Ointment, United States Pharmacopeia, 2009.

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